Time to study another variant of endothelial progenitor cells?

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This editorial refers to ‘Immune privilege of endothelial cells differentiated from endothelial progenitor cells’ by J. Ladhoff et al., pp. 121–129, this issue.

There is considerable interest in exploiting the functions of bone marrow-derived endothelial progenitor cells (EPC) for tissue repair and engineering.¹ However, with all the potential that EPC-based therapy may bring for vascular and organ repair, a major problem lies with the fact that patients who potentially need the most EPC-related cell therapy are those in which EPC levels and functions are the lowest, such as type II diabetic patients.² Allogeneic EPC therapy would thus be a suitable approach if it could be established that endothelial cells derived from EPC can survive within an allogeneic host. This may be a plausible proposal, as a novel study in Cardiovascular Research presents results demonstrating protection of allogeneic EPC-derived endothelial cells used in an aortic graft transplantation model.³ Although long-term survival of EPC in an autologous graft model has been shown,⁴ this new study shows survival of endothelial cells (EC) derived from EPC, up to 14 days post-transplantation, in a major histocompatibility complex (MHC)-mismatched allogeneic transplantation rat model. Ladhoff et al. used decellularized rat aortic grafts that were seeded with EPC-derived EC. They show that EPC-derived EC express low levels of MHC class I molecules and undetectable MHC class II molecules (except when the EC are stimulated with interferon-γ, in which case they still would express only very low MHC levels compared with mature rat aortic EC). These EPC-derived EC were also shown to be less susceptible to humoral immune recognition in cytotoxic T-lymphocyte-mediated killing assays.

Although the study of Ladhoff et al. adds a new perspective for EPC-based therapeutic approaches, it also brings with it yet another methodological variant to isolate and culture EPC, in this case, of the late-outgrowth EPC type. This contributes to the already debated problem regarding the identity of EPC and lack of standardized approaches for cell culture techniques used to isolate EPC.⁵,⁶ Different cell culture protocols and their variations can lead to different types of EPC cells. Although much of the early work on EPC focused on short-term protocols to isolate EPC in culture, it is known that the so-called ‘early EPC’ increase angiogenesis mainly by paracrine secretion of angiogenic factors whereas late-outgrowth EPC, also termed endothelial colony-forming cells, can directly participate in tubulogenesis and neovascularization.¹,⁷ In the present paper, Ladhoff et al. isolated and cultured the late-outgrowth type of EPC from rat for which, to my knowledge, there are no previous reports in the literature. It is known that late-outgrowth EPC do not originate from CD45⁺ cells and do not express the common leucocyte marker CD45.⁸ So, in order to obtain late-outgrowth EPC, the authors have used an original approach where they depleted CD45⁺ cells from rat peripheral blood mononuclear cells (PBMC) using magnetic beads coupled with anti-CD45 antibody. CD45-depleted PBMC were then cultured on fibronectin-coated dishes until outgrowing cell clusters appeared. Then, these clusters were picked using cloning rings and positively selected using PECAM-1 (CD31)-coupled magnetic beads. This cell fraction could then be cultured up to passage 25, therefore confirming proliferative capacity associated with the late-outgrowth type of EPC.⁹ These late-EPC cells were validated functionally as the authors showed that they can participate in in vitro tube formation but also in vessel formation in vivo using a rat skin transplantation model.³

The study of Ladhoff et al. thus generates numerous questions that would be exciting to investigate. For instance, does the procedure used to isolate late-outgrowth EPC from the rat result in cells with identical characteristics to those from the protocols typically used from human samples, that is, without immunoselection and, typically, grown on a collagen matrix?⁷ Also, it is known that murine and human endothelial cells differ in term of MHC class II expression and other molecules relevant for rejection.¹⁰ Would human late-outgrowth EPC-derived EC show the same immune privilege properties? In a study using human endothelial cells derived from cord blood late-outgrowth EPC (HCBEC), Suárez et al. reported that HCBEC behave very similarly to HUVEC in term of their ability to activate allogeneic memory CD4⁺ and CD8⁺ T cells, which suggested the need of finding MHC-matched donors in EPC-related therapy experiments.¹¹ It remains to be seen whether HCBEC obtained from this last report are identical to the late-outgrowth EPC-derived EC obtained using the methodological approach of Ladhoff et al.

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Moreover, although down-regulation of the expression or decreased function or the absence of MHC class I and II represent one type of mechanism of immune privilege, immune privilege in different organs may be generated or supported by a number of other mechanisms such as Fas ligand (CD95L) expression and the induced apoptosis of Fas<sup>+</sup> cells interacting with FasL-expressing cells. This pathway was not studied in the present paper and neither were the possible effects of systemic pro-inflammatory challenge in this aortic graft model, although the authors show in vitro that MHC expression by EPC-derived EC could be, at least to a low level, increased or induced by interferon-γ. In particular, it is known that Fas expression by EC may be increased by interferon-γ, which could then result in FasL-mediated apoptosis of endothelial cells. Another aspect that could be explored in future research is the level of functional reconstitution of the EPC-seeded graft vessel: for instance, does the remodeled vessel possess significant endothelium-dependent, NO-mediated relaxation capacity as was seen for ex vivo expanded EPC in an autologous graft model? It is known that the amount of NO production correlates strongly with patency rates of various vessel conduits used in bypass surgery. Autologous EPC-seeded grafts were shown to maintain patency up to 130 days. Longer term experiments using the model presented by Ladhoff et al. would thus be informative in this regard and would help to strengthen their very interesting observations.

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**References**